Kindly replace the paragraph beginning on page 2, line 33, to page 3, line 5, with the following text.

--Therefore, the present invention relates to a method of ligating the ds region end of a double-stranded DNA and the ss region end of another double-stranded DNA. The method comprises contacting, under the presence of a homologous recombinant protein, the ss region end of a double-stranded DNA and the ds region end of the other double-stranded DNA which comprises a sequence that is homologous to the abovementioned ss region nucleotide sequence to form a three-stranded DNA structural complex.--

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Kindly replace the paragraphs on page 3, lines 24-27 with the following text.

--Figure 1 shows a diagram of the reaction of the DNA cloning method by the Rec A three-strand formation reaction. DNA1 (SEQ ID NO:11) and DNA2 (SEQ ID NO:12) react according to the method of the invention to produce DNA(1+2) (SEQ ID NO:13).

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Figure 2 shows a diagram of the 5'RACE reaction by the Rec A three-strand formation reaction. cDNA1 and cDNA 2 react according to the method of the invention to produce cDNA fragment 1+2. cDNA1 contains a small stretch of 10 polyA nucleotides (SEQ ID NO:14)--

Kindly replace the paragraphs on page 4, lines 5-16 with the following text.

--Figures 7A and 7B show photographs of the gel electrophoretic pattern obtained in Example 5, in which the dependency of each reactant in the DNA end three-strand

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formation reaction using oligonucleotides was examined. Figure 7A shows an autoradiograph of the ethidium bromide stained agarose gel in 7B.

Figures 8A and 8B show photographs of the gel electrophoretic pattern obtained in Example 6, in which the sequence-orientation of oligonucleotides in the DNA end three-strand formation reaction using oligonucleotides, was examined. Figure 8A shows an autoradiograph of the ethidium bromide stainded agarose gel in 8B.

Figures 9A-9D show a photograph of the gel electrophoretic pattern obtained in Example 7, in which the heat stability of oligonucleotide sequence in the DNA end three-strand formation reaction, was examined. Figure 9A shows an autoradiograph of the ethidium bromide stained gel in Figure 9B. In Figures 9A and 9B, a labeled 60mer oligonucleotide was used. Figure 9D shows an autoradiograph of the ethidium bromide stained gel in Figure 9C. In Figures 9C and 9D, a labeled 40mer oligonucleotide was used.--

Kindly replace the paragraph on page 5, lines 15-30 with the following text.

--The present invention features a method of ligating the ss region end of a double-stranded DNA and the ds region end of another double-stranded DNA which comprises a sequence that is homologous to the nucleotide sequence of the aforementioned ss region (therefore, the other strand is complementary to said nucleotide sequence). This ligation can be presumed to occur simultaneously or sequentially at one point or at several positions in a linear DNA molecule, although ligation at two points is preferred. A circular or linear

DNA ligation is formed when ligation occurs at one point or several points in each end of one or several DNAs. Although it is not restricted, the formation of a circular DNA ligate is especially preferable. DNA ligates, also named as DNA constituents or DNA recombinants in this specification, means those constructed by a single or several double-stranded DNAs.--

Kindly replace the paragraph on page 6, lines 17-33 with the following text.

--Preferable are ligations between two kinds of DNA like those of the above-mentioned (ii) to (iii). In this case, the two ds region ends existing on DNA 1 and/or DNA 2 may have the same or different nucleotide sequences, and similarly, the two ss region ends may have the same or different nucleotide sequences corresponding respectively to the aforementioned ds region ends. "Corresponding respectively" means that a ds region end and an ss region end to be ligated, are in such a relationship that allows the formation of a three-stranded structure according to the present invention. Namely, it means that the nucleotide sequences of the sense-chain of the ds region end to be ligated, and the sense chain of the ss region end are homologous. For example, the chain corresponding to the single-stranded DNA upstream the double-stranded DNA of Figure 1, is named in this manner (i.e., sense chain) for convenience. Also the other chain down stream is named antisense chain. As for being "homologous" refer to the prior definition.--

Kindly replace the paragraph beginning on page 8, line 19, to page 9, line 15, with the following text.

--In the present invention, the aforementioned ds region end and ss region end are contacted under the presence of a suitable homologous recombinant protein within a liquid medium. The source of such a homologous recombinant protein (or a multi-functional protein that is involved in general recombination) is not questioned, and any protein may be used, as long as the aforementioned ds region end and ss region end can form a stable complex via said protein, when the said protein is present. Specific examples of such homologous recombinant proteins are, the Rec A protein of *E.coli* origin, multi-functional proteins encoded by the Rec A gene in heat-resistant bacteria (*Thermus thermophillus*) and other enteral bacteria, and already known Rec A-like proteins of *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Methylophilus methylotrophus*, *Vibrio cholerae*, *Ustilago maydis* and such origin. Yeast (*Saccharomyces cerevisiae*) and human-derived Rec-A like proteins are also encompassed in the aforementioned homologous recombinant protein. In the aspects of acquirability, stability and functioning, the *E.coli*-derived Rec A protein or a protein having a similar function is preferable for usage. For example, a modified protein of said protein origin or a fragment thereof can be used. As a modified protein, one that is a Rec A gene product created by site-specific mutagenesis of Rec A gene and such, and also comprising the amino acid sequence of the Rec A protein, in which one or more amino acids are deleted, replaced or added, and having a function equivalent to the Rec A protein, to form a complex comprising the aforementioned three- stranded DNA portion, can be

given. As a modified protein, with several amino acid deletions, a peptide or protein comprising the binding-domain to single-stranded DNA of Rec A protein, can be given. Examples of such peptides are, those given in the paper of Voloshin et al., Science, Vol. 272, 1996: 868-872. As understood by the above, the word "protein" in the present invention is used as a definition that encompasses peptides as well.--

Kindly replace the paragraph on page 10, lines 10-14 with the following text.

--The "contact" according to the present invention can be completed by incubating the mixture prepared as mentioned above at 4 to 54, preferably for 15 min at about 37, generally for 30 min. As a result, a DNA ligate (or DNA constituent) comprising at least one three-stranded structural portion is formed.--

Kindly replace the paragraphs beginning on page 10, line 37, to page 11, line 30, with the following text.

--The method of introduction can be suitably selected according to the starting material (vector) of the vector-derived DNA used as one of the DNAs. For example, when selecting an vector capable of auto-replicating inside *E.coli* as a vector or plasmid, and *E.coli* as the competent cell, introduction of the DNA constituent can be done using method such as electroporation method and calcium-treatment method. By culturing cells into which the above DNA constituent has been introduced, a circular DNA constituent can be amplified, in which the above-mentioned three-stranded structural region (when present)

had been converted to double-stranded structural region. This structural region is presumed to be in a state in which the nucleotide sequence corresponding to the above-mentioned ss region end has been removed from the abovementioned ds region end, and in which the ds region end and the ss region end is covalently-linked via a phosphodiester bond.

If ordinary methods used to introduce a gene into the aforementioned cells are followed, a circular DNA constituent will be more efficiently introduced into a cell than a linear DNA constituent. Therefore, when applying the method of the present invention using a PCR product as the DNA having the ds region end, those byproducts that are not correctly elongated by PCR will not be introduced to cells, since they do not form circular DNA constituents. The above-mentioned PCR product is obtained by a PCR reaction that uses as a primer, an oligonucleotide comprising nucleotide sequences corresponding respectively to the two types of ss region ends followed by each of the nucleotide sequences of the 5 or 3 end region of the gene to be ligated, and uses said gene as a template. Therefore, only the PCR products resulting from the correct amplification of the gene to be cloned by PCR, can be obtained by the method of the present invention.--

Kindly replace the paragraph on page 12, lines 12-20 with the following text.

--Examples of methods for converting a three-stranded structure to a double-stranded structure are, (1) the method that comprises transfecting the nucleic acid ligate obtained by the three-strand formation reaction into a prokaryotic or eukaryotic cell, and

converting the nucleic acid-ligated region including the three-strand structure into double-stranded DNA, or (2) the method of converting the nucleic acid-ligated region including the three-strand structure into double-stranded DNA using nucleic-acid modification enzymes, within a test tube.--

Sequence Listing

In compliance with 37 C.F.R. § 1.823(a), please insert the attached paper copy of the substitute Sequence Listing between the last page of the specification (page 27) and the first page of the claims. Please renumber the pages accordingly.

IN THE CLAIMS:

Kindly replace claims 1-5, 7, 10-12, 14-15 and 17-22 with the following amended claims.

1. (Amended) A method of ligating a double-stranded end of a double-stranded DNA and a single-stranded end of another double-stranded DNA, the method comprises contacting, in the presence of a homologous recombinant protein, the single-stranded end of said other double-stranded DNA and the double-stranded end of said double-stranded DNA, said double-stranded DNA comprises a sequence that is homologous to the nucleotide sequence of said single-stranded end to form a three-stranded DNA structural complex comprising said single-stranded end and said double-stranded end.

SUB D' CONT.

2. (Amended) The method of ligation of claim 1, wherein said three-stranded DNA structural complex is a circular DNA complex having a three-stranded structure in two positions, wherein said three-stranded structure is made by the ligation of:

a) a double-stranded DNA comprising a single-stranded region at both ends, and

b) a double-stranded DNA having at both ends a double-stranded region comprising sequences that are respectively homologous to said single-stranded nucleotide regions in a); or said three-stranded structure is made by the ligation of:

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c) a double-stranded DNA comprising a singe-stranded region at one end and a double-stranded region at the other end, and

d) a double-stranded DNA comprising a double-stranded region at one end having a sequence that is homologous to the nucleotide sequence of said single-stranded nucleotide region in a) and a single-stranded region at the other end comprising a sequence that is homologous to the nucleotide sequence of the double-stranded nucleotide region in a).

3. (Amended) The method of ligation of claim 2, wherein the nucleotide sequences of the two single-stranded regions in a) are mutually non-complementary.

4. (Amended) The method of ligation of claim 2, wherein the two single-stranded region ends in a) are within the same double-stranded DNA.

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5. (Amended) The method of ligation of claim 2, wherein one DNA from a) and b) or one DNA from c) and d) confers the ability of auto-replicating within competent cells.

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7. (Amended) The method of ligation of claim 1, wherein the nucleotide sequence of the single-stranded region is at least a 6mer.

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10. (Amended) The method of ligation of claim 1, wherein said method comprises a step of converting the three-stranded structure formed to a double-stranded structure.

11. (Amended) The method of ligation of claim 10, wherein the conversion of the three-stranded structure to a double-stranded structure is done by inserting the DNA complex comprising a three-stranded structure into cells.

SUB D2

12. (Amended) The method of ligation of claim 11, wherein the insertion of the DNA complex comprising a three-stranded structure into cells is done by electroporation.

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SUB D2

14. (Amended) The method of ligation of claim 1, wherein said method further comprises steps of converting the three-stranded structure into a double-stranded

SUB D2 Cont.

structure by treating the DNA complex having the three-stranded structure with endonuclease, inserting said treated DNA complex into cells, and culturing the transformant thus obtained to amplify DNA.

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15. (Amended) A DNA constituent comprising at least one three-stranded structure comprising a single-stranded region and a double-stranded region which comprises a sequence that is homologous to said single-stranded region.

17. (Amended) The DNA constituent of claim 15, wherein the constituent comprises two three-stranded structures, and the single-stranded nucleotide sequences forming these structures are mutually non-homologous.

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18. (Amended) The DNA constituent of claim 15, wherein each single-stranded region nucleotide is at least 6 mer.

19. (Amended) The DNA constituent of claim 15, wherein the three-stranded structure forms a complex with a homologous recombinant protein.

20. (Amended) The DNA constituent of claim 15, wherein one double-stranded DNA segment which is between two three-stranded structures confers the ability

of auto-replicating within competent cells, and the other double-stranded DNA segment comprises the whole or part of the gene to be cloned.

21. (Amended) A gene-cloning kit comprising the following components:

a) a DNA, which is a double-stranded DNA comprising a single-stranded region at both ends, wherein the nucleotide sequences of these single-stranded regions are mutually non-complementary, and furthermore comprises a DNA sequence which confers to the double-stranded region of said double-stranded DNA, the ability of auto-replicating within competent cells;

b) an oligonucleotide primer comprising as a part of the 5' end sequence, a sequence that is complementary to the one single-stranded region nucleotide sequence of (A), and is complementary to a part of the end of the sequence of the gene to be cloned, and;

c) an oligonucleotide primer comprising as a part of the 5' end sequence, a sequence that is complementary to the other single-stranded region nucleotide sequence of (A), and is complementary to a part of the other end of the sequence of the gene to be cloned.

22. (Amended) The kit of claim 21, wherein the nucleotide sequence of each single-stranded region is at least 6 mer.